Effect of alloxan diabetes on the fatty acid composition of the retina

Sidney Futterman, Ronald Sturtevant, and Carl Kupfer

The fatty acid composition of untreated alloxan-diabetic and normal rat retinal tissue was compared and the relative abundance of endothelial and mural cells in the retinal capillaries was estimated. Arachidonate and docosahexaenoate were decreased in diabetic retinal tissue; linoleate was increased. The changes observed indicate that insulin is required to maintain a normal rate of polyenoic fatty acid synthesis in retinal tissue. No selective loss of mural cells from the retinal capillaries could be found.

Key words: diabetes mellitus, alloxan, retinal capillaries, biosynthesis arachidonic acid, linoleic acid, docosahexaenoic acid, insulin.

In a previous study in which the incorporation of $^{14}$C from 1,3-$^{14}$C-malonyl-CoA into fatty acids by dog retinal tissue was examined,1 polyenoic fatty acid synthesis by retinal tissue was found to be depressed in alloxan diabetes. Additional support for the concept that the retina requires insulin is provided in this report by evidence that the fatty acid composition of the retina is altered in diabetic rats maintained without insulin for several months.

Methods

The methods used for analysis of the fatty acid composition of retinal tissue, preparation and examination of retinal blood vessels, and estimation of blood sugar were those previously employed.1,2 Diabetes was induced with alloxan (Fig. 1). Of 10 rats receiving alloxan, 7 became diabetic, with blood sugars remaining in excess of 300 mg. per cent, and were included in the study. A commercial pelleted rat diet and water were available continuously for both diabetic and control groups. The diabetic rats did not gain weight appreciably and all developed mature cataracts. They were maintained for 116 days without insulin and then painlessly put to death.

Results

The fatty acid composition of retinal tissue was altered in alloxan-diabetic rats (Table I). Diminished quantities of arachidonate and docosahexaenoate were present and the relative proportion of linoleate was increased 2.3-fold. Analysis of the relative abundance of fatty acids by chain length, after hydrogenation of the methyl esters, indicated that in diabetic retinal tissue the relative proportion of $C_{20}$ and $C_{22}$ fatty acids was decreased and $C_{16}$ and $C_{18}$ fatty acids increased.

The diet which the rats received contained 4 per cent fat. Analysis of the diet for fatty acids indicated that the principal fatty acids in the pellets were palmitate,
Table I. Fatty acid composition (mole per cent) of diabetic and normal rat retina*

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Carbon chain</th>
<th>Normal (6)</th>
<th>Diabetic (6)</th>
<th>Hydrogenated fatty acids</th>
<th>Carbon chain</th>
<th>Normal (5)</th>
<th>Diabetic (6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palmitate</td>
<td>16:0</td>
<td>19.6 ± 0.3</td>
<td>25.6 ± 0.8</td>
<td>Palmitate</td>
<td>16:0</td>
<td>20.7 ± 0.3</td>
<td>25.2 ± 0.7</td>
</tr>
<tr>
<td>Stearate</td>
<td>18:0</td>
<td>25.0 ± 0.7</td>
<td>27.5 ± 0.1</td>
<td>Stearate</td>
<td>18:0</td>
<td>35.9 ± 0.5</td>
<td>41.5 ± 0.8</td>
</tr>
<tr>
<td>Oleate</td>
<td>18:1</td>
<td>9.8 ± 0.2</td>
<td>12.7 ± 0.5</td>
<td>Arachidate</td>
<td>20:0</td>
<td>8.6 ± 0.2</td>
<td>8.6 ± 0.2</td>
</tr>
<tr>
<td>Linoleate</td>
<td>18:2</td>
<td>0.7 ± 0.03</td>
<td>1.6 ± 0.2</td>
<td>Behenate</td>
<td>22:0</td>
<td>33.7 ± 0.1</td>
<td>24.7 ± 1.03</td>
</tr>
<tr>
<td>Arachidonate</td>
<td>20:4</td>
<td>9.0 ± 0.1</td>
<td>7.6 ± 0.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Docosahexaenoate</td>
<td>22:6</td>
<td>36.0 ± 1.1</td>
<td>25.0 ± 2.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mural cell count†</td>
<td>97 ± 2.2</td>
<td>96 ± 4.0</td>
<td>(4)</td>
<td>(4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Minor components, with the exception of linoleate, have not been included; values given are means ± standard errors of the means. The number of animals analysed in each group is indicated in parentheses. Approximately 0.41 μmole of fatty acids were obtained from normal rat retinas, and 0.34 μmole from diabetic retinas.

†Number of mural cells per 100 endothelial cells in retinal capillaries.

Fig. 1. Mean weight gain of 7 alloxan-diabetic and 10 normal male Sprague-Dawley rats. Rats in the diabetic group each received an intraperitoneal injection of 10 per cent alloxan monohydrate (300 mg. per kilogram) on day 10 and day 11, as indicated by arrows. Value given are means ± standard errors of the means.

22 per cent; stearate, 9 per cent; oleate, 30 per cent; linoleate, 34 per cent; and linolenate, 4 per cent. The diet did not contain detectable amounts of either arachidonate or docosahexaenoate. It was apparent, therefore, that these fatty acids in retinal tissue were derived by the desaturation and chain elongation pathways of linoleate and linolenate metabolism and were not of dietary origin.

In contrast to observations of decreased numbers of mural cells in diabetic human and canine retinal capillaries, 2 4 no evidence for selective loss of mural cells could be found in retinal capillary preparations from alloxan-diabetic rats (Table I).

Discussion

When the utilization of "C-malonyl-CoA for polyenoic fatty acid synthesis by retinal tissue was found to be impaired in alloxan diabetes, 1 it was not known whether the defect was sufficient to change the composition of the retina. It is now clear that the defect in polyenoic fatty acid biosynthesis does result in an altered fatty acid composition of the retina. In the course of turnover of structural lipids in the retina, it appears that there is incomplete replacement of polyenoic fatty acids in the severely diabetic state, with some substitution of saturated and monoenoic fatty acids. The changes in fatty acid composition observed in the alloxan-diabetic rat retina provide evidence that retinal tissue requires insulin.

Retinopathy has not been produced in the alloxan-diabetic rat. 5 6 Failure to demonstrate mural cell loss in this study may possibly be attributable either to insufficient time being allowed for this to occur or to significant species differences in the metabolism of mural cells.

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REFERENCES